

AMENDMENT TO THE SPECIFICATION

Please note that the **additions** are **both underlined and bold-faced** as the original specification contains text that is underlined or bold-faced.

Please replace pages 44-59 (containing a sequence listing) with the new sequence listing enclosed herewith.

Please amend the paragraph starting at page 28, line 18 as follows:

-- Fig. 2: Full length cDNA sequence (**SEQ ID NO:1**) of a plant GnTI from potato (*Solanum tuberosum* L.) and amino acid sequence deduced therefrom (**SEQ ID NO:2**). By way of example, the complete cDNA of the membrane anchor containing *GntI* isoform from potato leaf tissue (A1) is illustrated. The EcoRI/NotI linkers at the 5' and 3' ends of the cDNA are highlighted by bold letters, the binding sites of the degenerate oligonucleotides used for obtaining the RT-PCR probe are underlined. In contrast to already published animal GnTI sequences, the protein sequence derived from the potato cDNA clones contains a potential N-glycosylation site: Asn-X (without Pro)-Ser/Thr, which is indicated by an asterisk. The region of the membrane anchor is highlighted in italics (aa 10 to 29). The start of the isoform (A8), which is potentially located in the cytosol, is indicated by an arrow.--

Please amend the paragraph starting at page 29, line 6 as follows:

-- B, Comparison of the derived amino acid sequences of different plant *GntI*-cDNA clones. A_Stb-A1 (**SEQ ID NO:2**), GnTI from potato leaf; B_Ntb-A9 (**SEQ ID NO:4**), GnTI from tobacco leaf (A9); C_At看-Full (**SEQ ID NO:6**), GnTI from *Arabidopsis thaliana*. Identical aa are highlighted in black, similar aa in light grey.--

Please amend the paragraph starting at page 31, line 16 as follows:

-- Total RNA was isolated from potato and tobacco leaf tissue, and cDNA fragment of about 90 bp were amplified by means of RT-PCR in combination with degenerate primers

(procedure analogous to ref. 31), which were derived from conserved amino acid regions of known GnTI sequences from animal organisms (sense primer 1* (**SEQ ID NO:7**), 5'-TG(CT) G(CT)I (AT) (GC) I GCI TGG (AC)A(CT) GA(CT) AA(CT) -3'; antisense primer 3* (**SEQ ID NO:8**), 5' -CCA ICC IT(AG) ICC (ACGT)G(CG) (AG)AA (AG)AA (AG)TC-3'; 30 pmol of each primer per 50 μ l PCR assay at an annealing temperature of 55°C and 45 cycles). Following gel elution, the ends of the PCR products were repaired (i.e. blunt ended using DNA polymerase I and phosphorylated using T4 polynucleotide kinase) and clones into the EcoRV restriction site of pBSK (Stratagene). By comparison with known GnTI sequences between the primers (arrows), the identity of the derived amino acid sequences from the potato and tobacco RT-PCR products could be confirmed as being homologous; \Rightarrow Q(R/M)QFVQDP(D/Y)ALYRS (**SEQ ID NO:9**) \Leftarrow (homologous aa are underlined). Of one clone each, radiolabelled probes were synthesized by means of PCR (standard PCR assay using degenerate primers as above, nucleotide mixture without dCTP, but instead with 50 μ Ci α -³²P-dCTP [>3000 Ci/mMol]), and different cDNA libraries were screened for *GntI* containing clones using the corresponding homologous potato or tobacco probes, respectively (procedure analogous to ref. 31; the stringent hybridization conditions have already been described in the text above). The cDNA libraries were prepared from mRNA of young and still growing plant parts (sink tissues). Following cDNA synthesis and ligating EcoRI/NotI adaptors (cDNAsynthesis kit, Pharmacia) EcoRI compatible lambda arms were ligated, those packaged and used to transfect *E. coli* XL1 Blue cells (Lambda ZAPII cloning and packaging system, Stratagene). Following amplification of the libraries, one full-length *GntI* clone each was isolated from a potato leaf sink library (A1 according to Fig. 2 and SEQ ID NO: 1) and a tobacco leaf sink library (A9 according to SEQ ID NO: 3), as well as two additional clones from a tuber sink library (A6, A8). The deduced GnTI amino-acid sequences contain a potential N-glycosylation site, Asn-X (without Pro)-Ser/Thr, in contrast to those of animals. One of the tuber *GntI* cDNA sequences carries stop condons in all three reading frames in front of the first methionine (A8). The coding region shows high homology to the longer tuber clone (A6) (only 2 aa substitutions), but displays a completely different 5' non-translated region. Furthermore, the membrane anchor characteristic for the Golgi enzyme

is missing, so that this GnTI isoform might be located in the cytosol. Sequence comparisons carried out by means of the gap or pileup option, respectively, and the box option of the gcg software package (J. Devereux, P. Haeberli, O. Smithies (1984) Nucl. Acids Res. 12: 387-395) indicate, that the deduced plant GnTI amino-acid sequences exhibit only 30-40% identity and 57-59% similarity to those of animal organisms (Fig. 3A), while they are highly homologous among each other (75-90% identity, Fig. 3B). --

Please amend the paragraph starting at page 32, line 39 as follows:

-- The procedure in the case of *Arabidopsis thaliana* was analogous, wherein for the preparation of a specific probe first a partial *GntI* sequence was amplified by RT-PCR using *GntI* sense primer 4A (**SEQ ID NO:10**; 5'-ATCGGAAAGCTTGGATCC CCA GTG GC(AG) GCT GTA GTT GTT ATG GCT TGC -3'; HindIII restriction site underlined, BamHI printed in bold) and antisense primer 3*, as defined above. First, a 5'-incomplete cDNA clone was isolated from a phage library (Lambda Uni-Zap) using this probe. By means of a vector insert PCR, the missing 5'-terminus was amplified from another library (via ~~an~~ a unique SpeI restriction site in the 5' region) and assembled to yield a full-length cDNA sequence. The nucleic acid sequence determined by means of sequencing is listed in SEQ ID NO: 5.--

Please amend the paragraph starting at page 34, line 5 as follows:

-- Into the SalI restriction site of the polylinker region (corresponding to the one of pUC18) of plant expression vector pA35 (ref. 29), a NotI linker was introduced subsequently to the fill-in of the ends (=pA35N), and the complete A1-*GntI*-cDNA (nucleotides 9 to 1657, according to the cDNA in Fig. 2) was inserted into pA35N *via* NotI (sense construct pA35N-A1s and antisense construct pA35N-Alas, respectively). The expression cassettes of the sense and antisense construct, respectively, were isolated via the terminal restriction sites (filled-in NcoI restriction site, partial post digestion with HindIII) as a fragment of about 2410 bp and inserted into the EcoRI (filled-in) and HindIII restriction sites of the binary vector pBin19 (Ref. 30) (=pBin-35-A1s and pBin-35-Alas, respectively). The EcoRI restriction site of the vector is restored by fusion with the equally

filled-in NcoI restriction site of the fragment. By means of a standard PCR assay (sense primer (SEQ ID NO: 11): KS sequencing primer (Stratagene) extended for PCR, 5'-GGC CCC CCC TCG AGG TCG ACG GTA TCG-3'; antisense primer (SEQ ID NO: 12): 5'-GGGCCTCTAGACTCGAG AGC (CT)AC TAC TCT TCC TTG CTG CTG GCT AAT CTT G-3', XbaI restriction site underlined, XhoI restriction site in italics), there was additionally amplified a 5'-fragment of the *GntI* cDNA at an annealing temperature of 50°C (nucleotides 9 to 261, according to the cDNA in Fig. 2 and SEQ ID NO: 1). The PCR product was digested with XbaI (within the antisense primer) and NotI (within the 5'-linker of the cDNA), isolated as a fragment of about 260 bp and cloned into pA35N (=pA35N-A1-short). The expression cassette of the short antisense construct was also inserted into pBin19 (=pBin-35-A1-short) as a EcoRI/HindIII fragment (about 1020 bp).--

Please amend the paragraph starting at page 35, line 41 and continuing into page 36, line 30 as follows:

-- Recombinant GnTI carrying 10 additional N-terminal histidine residues (His-tag) was produced in *E. coli* by means of the pET system (Novagen) and purified by metal-chelate affinity chromatography. A cDNA fragment comprising nucleotides 275-1395 of the potato *GntI* cDNA (corresp. to aa 75-446, Fig. 2 and SEQ ID NO: 1 and 2, respectively) was amplified by standard PCR (annealing temperature of 50°C, 30 cycles, ref. 31) (sense primer *GntI*-5' fus (SEQ ID NO: 13): 5'-CATGGATCC CTC GAG AAG CGT CAG GAC CAG GAG TGC CGG C-3'; antisense primer *GntI*-3' stop (SEQ ID NO: 14): 5'-ATCCCGGGATCCG CTA CGT ATC TTC AAC TCC AAG TTG-3'; XhoI and BamHI restriction sites, respectively, are underlined, stop codon in italics), and inserted into vector pET16b (Novagen) (=pET-His-A1) via the restriction sites of the synthetic primer (5'-XhoI-*GntI*-BamHI-3'). Following propagation and analysis in *E. coli* XL1-Blue (Stratagene) the construct was stored as a glycerol culture. Competent *E. coli* BL21 (DE3) pLysS cells (Novagen) were transformed with pET-His-A1 for overexpression. Addition of IPTG (Isopropyl-1-thio-β-D-galactopyranoside, at 0.5-2 mM) to a BL21 culture in logarithmic growth phase, initially induces the expression of T7

RNA polymerase (from the bacterial chromosome), and thus, also the expression of the recombinant fusion protein under control of the T7 promoter in pET vectors (Novagen). By means of metal-chelate chromatography using TALON matrix (Clontech), recombinant potato GnTI was purified from induced BL21:pET-His-A1 cells under denaturing conditions *via* its His-tag (manufacturer's protocol, Novagen), and the preparation was verified with respect to homogeneity by means of SDS-PAGE.--